Profiling of viral safety of biopharmaceuticals produced from milk from Danish dairy cattle

Christensen, Laurids Siig

Publication date:
2007

Document Version
Publisher's PDF, also known as Version of record

National Food Institute

Tecnical University of Denmark DTU

Profiling of viral safety of biopharmaceuticals produced from milk from Danish dairy cattle

Profiling of viral safety of biopharmaceuticals produced from milk from Danish dairy cattle

By

Laurids Siig Christensen
Senior Scientist, MSc & DMSc
National Food Institute
Technical University of Denmark
Moerkhoej Bygade 19
DK-2860 Soeborg
Denmark

The present risk profile was requested by a medical company developing a biopharmaceutical product. All references to the company, the product and its manufacturing process have been erased from the present version of the report.

May 2007

Laurids Siig Christensen

Profiling of viral safety of biopharmaceuticals produced from milk from Danish dairy cattle

1st edition, 1st circulation, May 2007
Number printet: 50

ISBN: 978-87-92158-04-8

The report is available at www.food.dtu.dk

National Food Institute
Technical University of Denmark
Moerkhoej Bygade 19
DK-2860 Soeborg
Denmark

Tel: +45 72 34 70 00
Fax: +45 72 34 70 01

Contents

List of abbreviations

AI	Artificial insemination
BSE	Bovine spongiform encephalopathy
BVDV	Bovine viral diarrhoea virus
EBL	European bat lyssavirus
EMCV	Encephalomyocarditis
FMD	Foot-and-mouth disease
IBR	Infectious bovine rhinotracheitis
HEV	Hepatitis E virus
OIE	Office Intenational Epizootic
Orf	Contagious ecthyma
RVFCA	Regional Veterinary and Food Control Authority
TBE	Tick borne encephalitis
TSE	Transmissible spongiform encephalopathy
vCJD	variant Creutzfelt-Jacobs disease
SHV-1	Suid herpesvirus 1
WHO	World Health Organisation
WNV	West Nile virus

Summary

As a result of survival of the fittest-driven evolution predators generally do not acquire life-threatening infections from their prey. Biopharmaceuticals produced for humans from horses, pigs, cattle and poultry therefore in general can be considered of low risk since only few pathogens of these species with zoonotic potential can be identified and no adverse infection epidemics have emerged during the 19th and 20th centuries as result of the extensive use of biopharmaceuticals produced from our prey. Biopharmaceuticals produced from milk from Danish dairy cattle in addition can be considered highly safe with regard to known viruses based on the considerations of i) the high zoosanitary status of Denmark in general and of Danish dairy cattle in particular, ii) the measures currently taken to maintain this zoosanitary status, iii) the population containment of domestic animals in Denmark in general and Danish dairy cattle in particular, and iv) the measures currently taken to ensure the hygiene of milk for human consumption, in particular the mandatory process of pasteurization.

Prions (transmissible spongiform encephalopathies) by WHO and OIE are not considered a potential contaminant of milk from cows and as bovine spongiform encephalopathy (BSE), in addition, is nearly eradicated in Denmark as documented by the surveillance programme, prions are not considered a safety issue in biopharmaceuticals produced from Danish dairy milk.

Addressing the issue of emerging viral pathogens, it is added that a combination of inactivation and removal steps of the manufacturing process should still be included and validated for the capacity to provide a satisfactory elimination of relevant model viruses.

Introduction

Biopharmaceuticals from domestic animals have been developed and used for humans since the principle of vaccination was discovered by Edward Jenner (1798) who used vesicular material from the teats of a cow to develop protection against smallpox in humans. Among other major revolutions in medical treatment of humans that can be mentioned were the production of hyper-immune serum in horses against toxins such as diphtheria and tetanus and the production of insulin from porcine and bovine pancreas. By practice during a couple of centuries it was shown that the exposure to zoonotic agents from these source animals was not a significant hazard. Yet, the advent of genetically modified organisms and the emergence in our food chain of infectious agents such as prions as well as the emergence of new viruses during the past 40 years stresses the need to currently asses the risk of contamination with infectious agents in biopharmaceuticals.

The present report will summarize viruses and prions relevant as a potential contaminant in Danish dairy milk and assess the risk of their presence in non-homogenized, pasteurized skimmed milk prepared in compliance with current EU and national regulations for manufacturing of milk for human consumption.

Viral infections of cattle with zoonotic potential

The term “zoonotic virus” refers to viruses with a recognized animal reservoir with human pathogenic potential as well as environmental viruses with recognized or unrecognized reservoir species to which domestic animals - as also humans might be exposed.

A variety of zoonotic viruses exists as reviewed by Acha and Szyfres (2003) while relatively few viruses are known in cattle to cause mastitis, directly or indirectly, or to cause superficial affection of the teats or udder as reviewed by Wellenberg et al. (2002). Only few of the zoonotic viruses are known to be pathogens in cattle and only few of these are known to be relevant in Europe in general and in northern Europe and Denmark in particular. The zoonotic viruses which by any means are relevant in relation to a potential contamination of milk from Danish dairy cattle are reviewed in the following.

Cowpox virus

Cowpox virus is a member of the *Poxviridae* family, genus *Orthopoxvirus* (reviewed by Moss, 2001, and Esposito and Fenner, 2001). Members of the family have unique envelope structures and a double-stranded DNA genome. The virus is highly resistant to drying and may retain infectivity for months in dry environments. It is sensitive to temperatures >40^{o}C, pH extremes and detergents. Orthopoxvirus infections are often associated with severe morbidity and high mortality in their reservoir species.

Cowpox virus gained its fame when Edward Jenner (1798) conducted a series of experiments that showed that inoculation with material (vaccinia) isolated from the teats of a cow or hoofs of a horse suffering from the benign condition of cowpox provided immunity in humans against the smallpox (variola). This conclusion marked the entrance to the era of vaccination. In spite of its name, the natural reservoir of cowpox virus is not cattle but rodents (Marennikowa, 1977), and the benign symptoms of pox occasionally seen in cats are caused by cowpox virus acquired from their prey. Cats probably serve as the most frequent vector of transmission to humans. Cowpox virus is characterized by a wide range of species susceptible to infection.

The recent identification of the first case of cowpox in Denmark revealed that the virus is present in Denmark but the sero-prevalence in Danish cats revealing an exposure of <5% of cats also lead to the conclusion that the prevalence in Denmark is very limited (Christensen et al., 2005; Christensen, 2005). The prevalence of cowpox virus in cows is debated. It was concluded by Bollinger (1877) that cases of cowpox in cattle could always be attributed to spill-over from vaccinated cattle handlers. More recent sero-prevalence screenings in various parts of the world, however, reveal that cattle occasionally might become infected with cowpox virus or a related member of the *Orthopoxviridae*.

Pseudocowpox virus and other parapoxviruses

Pseudocowpox virus belongs to another genus, *Parapoxvirus*, of the *Poxviridae* family (reviewed by Moss, 2001, and Esposito and Fenner, 2001) and share many biological and physical characteristics with cowpox virus. Serological cross protection between members of the two genera is not seen and parapoxviruses can be further distinguished from orthopoxviruses by another unique envelope structure. Parapoxvirus infections are always benign in their reservoir animal species. Pseudocowpox virus causes milkers nodules in humans, considered a benign occupational hazard of cattle handlers.

Papular stomatitis virus is a close relative of pseudocowpox virus and can be differentiated only by its clinical manifestation. Pseudocowpox virus cause lesions on the teats and papular stomatitis virus causes stomatitis in calves. Manifestations in humans are identical.

Contagious ecthyma (orf) virus is another close relative of pseudocowpox virus and a benign infection of sheep and goats causing pustular dermatitis or stomatitis in young animals. By contact with infected sheep or goats it can cause single or multiple lesions on the fingers or hands of humans, rarely spreading to other parts of the body. According to the records of the diagnostic laboratory, orf virus is a widely disseminated infection among sheep in Denmark and incidental spill-over to cattle can not be excluded.

Pseudocowpox virus is ubiquitous in Denmark causing epidemics of teat lesions in dairy cattle farms, the infection being transmitted by the cups of milking machines (Andersen and Skovgaard, 2004). Although the lesions are superficial on the udder and teats contamination of milk is likely. Thus, pseudocowpox virus is a most relevant agent and a suitable model for other members of the *Poxviridae* to be used for assessment of efficacy of inactivation/removal by the processing of biopharmaceuticals from dairy milk.

Rabies virus

Rabies virus belongs to the *Rhabdoviridae* family, genus *Lyssavirus*, of the order *Mononegavirales* (single-stranded, negative sense RNA viruses) (reviewed by de Mattos et al., 2001). Members of this family are enveloped and sensitive to temperatures >56^{o}C, lipid solvents, ultraviolet light and X-radiation.

The genus *Lyssavirus* consists of 7 species (plus some with very limited geographical dissemination) among which 3 species, sylvatic (classical) rabies virus, European bat lyssavirus 1 (EBL-1) and European bat lyssavirus 2 (EBL-2) are found in Europe.

The natural reservoir of sylvatic rabies virus in West Europe is the fox. The virus can be transmitted by saliva through wounds to a very wide range of susceptible animal species, inevitably with a fatal outcome.

Sylvatic rabies virus is an exotic (not present) and notifiable infection in Denmark. Rabies was subject to eradication in Denmark during the 20'ieth century and for a decade was only occasionally observed in the border areas to Germany. The last case of rabies was found in the border area in a cow in 1982 and since then the infection has been eradicated in Northern Germany by bait vaccination of foxes.

EBL has been recorded in bats in Denmark since 1985 with varying incidence and apparently is widely disseminated. EBL infection was observed also in sheep (Ronsholt, 2002) and a stone marten (Müller et al., 2001). Serological evidence furthermore suggests that cats very rarely acquire the infection (Tjørnehøj et al., 2004). Experimental infections reveal that a variety of animals are susceptible to infection (Fekadu et al., 1988; Vos et al., 2004a; Vos et al., 2004b). Yet, EBL is known to be much less virulent than sylvatic rabies virus and transmission to other animals than bats is known to be very rare. EBL infection is notifiable in Denmark.

A majority of viruses emerging during the past 40 years such as the henipaviruses, the filoviruses and seal distemper virus are members of the *Mononegavirales*. For these reasons, an assessment of the efficacy of inactivation/removal of a model for lyssavirus by the processing of biopharmaceuticals from dairy milk is relevant.

Tick borne encephalitis virus

Tick borne encephalitis (TBE, Russian spring-summer encephalitis, Central European tick borne encephalitis and Far Eastern tick borne encephalitis) virus is a member of the genus *Flavivirus* of the *Flaviviridae* family (reviewed by Burke and Monath, 2001) and a member of the complex including also Louping ill virus, Powassan virus, Kyasanur Forest disease virus, Omsk haemorrhagic fever virus and Langat virus. Members of the *Flaviviridae* family are enveloped and have a single-stranded positive sense RNA genome. Flaviviruses generally are inactivated at acidic pH, temperatures >40°C, organic solvents, detergents, ultraviolet light and gamma-irradiation. Yet, TBE virus exhibits a significant resistance to acid pH and transmission in unpasteurized goat milk has been observed (Gresikova et al., 1975).

TBE is found in all parts of the world including Denmark at the island of Bornholm. The infection is found mainly in wooded areas and pastureland bordering such areas. Serological screenings in various parts of the World have revealed exposure of a substantial proportion of goat and cattle populations in such areas. The life cycle of the virus involves ticks and small mammals, in particular rodents. The tics which might involve various species of *Ixodes* and *Haemophysalis* are the vectors of transmission to domestic animals and to humans alike.

Clinical symptoms in domestic animals and humans involve affection of the central nervous system. Central European TBE leads only to clinically apparent disease in 2% of the cases, and fatality rates in humans in those cases are 1–2% .

Human cases of TBE has never been found in Denmark except from the island of Bornholm, and serological screening in the late 50'ies on roe deer revealed that the infection in Denmark was restricted to Bornholm. A similar screening more recently suggested the infection to have become prevalent also in other parts of Denmark (Skarphedinsson et al., 2005) but this has not been confirmed by any clinical case in humans and the specificity of the assay used in the screening is questioned.

West Nile virus

West Nile virus (WNV) is another member of the genus *Flavivirus* of the *Flaviviridae* family (reviewed by Burke and Monath, 2001) and a member of the complex including also St. Luis, Murray Valley, Japanese and Rocio encephalitis viruses. WNV is sensitive to methods of physical and chemical inactivation.

WNV is widespread in Africa and previously was only recognized as a disease entity in Africa. However, serological screenings have suggested that the infection is widespread in Asia as well as in Europe, and given the concern that resulted from the introduction of WNV to the USA in 1999, islands of clinical cases were reported on various locations in West Europe. In endemic areas the majority of the population are infected at a young age and, hence, immune in adulthood. On the North American continent WNV rapidly spread from the primary focus of New York throughout the continent with the exception of the northernmost provinces of Canada and Alaska.

Multiplication of WNV involves a complex interplay between members of a variety of mosquito species in which WNV may persist from one season to the next, members of a variety of bird species and members of a variety of mammal species among which disease has most often been reported in horses. Birds are considered the reservoir, mosquitoes are considered the vector and mammals are considered accidental hosts not essential in the life cycle of the virus. Yet, WNV seem to be very capable of adapting to the species constituting highly diverging ecosystems, and transmission mammal-to-mammal has been observed among farmed alligators as well as among various bird species (ProMED mail Post, 2003; McLean et al., 2001).

WNV infection is notifiable in Denmark. It has never been reported in Denmark but serological screenings have not been conducted to exclude its presence.

Encephalomyocarditis virus

Encephalomyocarditis virus (EMCV) (reviewed by Acha and Szyfres, 2003) is a member of the genus *Cardiovirus* of the family *Picornaviridae*. Members of this family are non-enveloped and have a single-stranded, positive-sense RNA genome. EMCV is resistant to solvents and detergents and moderately resistant to acidic pH.

EMCV is found in all parts of the world and has been detected in a large variety of animal species including cattle as well as in birds. Swine is the animal in which it is most often detected. The principal reservoir is not known but rodents are suggested candidates. Transmission routes to domestic animals as well as to humans are also not known, and intra-species transmission has not been demonstrated. The symptoms in humans are generally unspecific but may involve the central nervous system. No deaths have been reported. Serological screenings in other regions of the World imply an unappreciated exposure of major proportions of populations.

The clinical picture in swine is highly variable, but encephalitis and sudden death are characteristic symptoms of EMCV infection.

EMCV infection is notifiable and has never been diagnosed in Denmark. Based on the clinical picture seen elsewhere, it is the general conception that it is unlikely that the infection could be present unattended.

Hepatitis E virus

Hepatitis E virus (HEV) (reviewed by Purcell and Emerson, 2001) has not been finally classified but is a non-enveloped, positive-sense, single-stranded RNA virus. HEV is assumed to be resistant to organic solvents and pH extremes.

HEV has been found in all parts of the World where it has been looked for and in a variety of domestic and wild ranging animal species. HEV is ubiquitous in humans and the major cause of sporadic hepatitis in regions where the infection is endemic. Mode of transmission is fecal-oral and water-borne epidemics have been reported. Since the age-related peaks of disease are found in early childhood and adolescence a sexual involvement in transmission has been suggested. The possibility of transmission from animal reservoirs is a controversial issue and data are conflicting. Yet, this has to be considered a possibility.

During epidemics the majority of patients experience jaundice, anorexia and hepatomegaly. In addition, abdominal pain, nausea, vomiting and fever are typical symptoms. The clinical picture can not be differentiated from that of hepatitis A virus and, likewise, no chronic infection is established.

The sero-prevalence of HEV in domestic animals in Denmark has not yet been investigated.

Prions

Prions causing transmissible spongiform encephalopathies (TSE) of relevance include scrapie and bovine spongiform encephalopathy (BSE, mad cow disease). BSE most likely originated from scrapie due to the use of meat and bone marrow from sheep as feed for cattle, in particular in the UK. BSE is assumed by February 2007 to have caused the death of 198 humans suffering from variant Creutzfelt-Jacobs disease (vCJD) originating from BSE infection (ProMED, 2007). Scrapie is not considered a risk for humans, and milk and milk products from cows are considered free of BSE based on infection experiments and epidemiological data as stated also by the OIE terrestrial Animal Health Code 2005 article 2.3.13.1, 1a. In addition, both EMEA (2003) and WHO (2003) concluded that milk of dietary quality is to be considered safe raw material for biopharmaceuticals with regard to TSE's. Yet, the presence in Denmark of both infectious agents is summarized in the following based primarily on the report from The Danish Veterinary and Food Administration (Anon., 2006).

Denmark has had a full surveillance program complying with the TSE legislation by the EU commission in EU Regulation 999/2001.

Classical (infectious) scrapie has never been found in Denmark. Due to the program of active and passive surveillance Denmark is considered free of classical scrapie.

BSE has been reported in all EU member states. In Denmark a total of 14 cases in cattle have been identified since February 2000. In addition, 3 cases were found in cattle born in Denmark and exported. The last case in Denmark was reported in September 2005. Prior to that all animals were culled on farms, where a case of BSE was found. The two cases in 2004 and 2005, respectively, were in animals 168 and 113 months of age, while the cases reported during 2000 and 203 were in the range of 39 to 90 months of age. No animal born in 2000 or thereafter has been found with BSE assumingly reflecting that the source of exposure in Denmark has been eliminated due to tightening of rules to comply with the ban on feeding ruminant meat and bone marrow to ruminants as established in 1990.

Denmark has applied for status as "a country with a controlled BSE risk" according to the OIE Terrestrial Animal Code 2005 Article 2.3.13.4. The National Veterinary Institute has made a prognosis indicating that, as January 1st, 2006, the total number of new BSE cases for the period of 2006-2010 will be 0,14. Thus, based on the results of the extensive surveillance programme it is clear that eradication of infectious BSE in Denmark is nearly completed.

Zoosanitary status of Denmark

The high zoosanitary status of Denmark together with the other Nordic countries is probably not exceeded in any other part of the World.

Denmark constituting a peninsula to Germany and a cluster of islands historically was free of or had a low incidence of a variety of infections found in Central Europe. In addition, Denmark has a long tradition for control and eradication of infectious diseases in domestic animals as summarised by the Danish Veterinary and Food Administration (Anon., 2007). Examples are the eradication of bovine tuberculosis, bovine brucellosis the last case of which in Denmark was seen in 1962, foot-and mouth disease, sylvatic rabies the last case of which was seen in 1982, Aujeszky's disease, Enzootic bovine leukosis, bovine herpesvirus 1 (infectious bovine rhinotracheitis (IBR) virus), and bovine viral diarrhoea virus (BVDV) nearly completed.

Monitoring and maintenance of the zoosanitary status of Denmark

The zoosanitary status of Denmark is subject to legislation as described at

http://www.uk.foedevarestyrelsen.dk/AnimalHealth/Prevention_control_animal_diseases/forside.htm

It is controlled and maintained by a variety of measures governed by the Ministry of Family and Consumer Affairs and/or by the producer organisations and/or by the discretion of the individual farmer. Among the principles of significance in the present context are the following:

Disease surveillance in cattle

The Danish Animal Health Act of 2004 is the legislative basis for the notification procedure and defines which animal diseases are notifiable in Denmark as summarised by the Danish Veterinary and Food Administration (Anon., 2007).

The notifiable animal diseases are divided into two groups, i.e. list 1 and list 2. Suspicion of a disease on list 1 shall immediately be notified to the Veterinary Department, whereas notification of a disease on list 2 is only mandatory after confirmation of the disease. The Danish list 1 notifiable diseases include all former OIE list A diseases as well as several zoonotic diseases. A farmer is obliged to immediately call a veterinarian if the farmer suspects a notifiable disease. The veterinarian shall immediately notify the Regional Veterinary and Food Control Authority (RVFCA) if the veterinarian suspects a disease on list 1. If the RVFCA cannot rule out the suspicion of a list 1 disease the farm is

placed under official surveillance and test material is collected and dispatched to laboratory investigation. All suspicions of notifiable diseases are immediately announced on DVFA's website to increase farmers and veterinarian's awareness of the potential presence of a notifiable disease.

Active surveillance programmes for cattle include serological testing for a variety of infectious agents of bulls at artificial insemination (AI) centres, slaughtered animals and cattle for export as well as bulk milk from dairy farm tanks. The testing programs are specified in Bekendtgørelse Nr. 305 (2000), 306 (2000), 112 (2005), 28 (2006), 29 (2006) and 30 (2006).

- Bulls at AI centres are tested when introduced and once every year for IBR, BVDV, tuberculosis, and brucellosis.
- Slaughtered animals are tested for IBR, BVDV, enzootic bovine leucosis, *Salmonella* Dublin, tuberculosis and brucellosis
- Cattle for export are tested depending of country of destination and in most cases for tuberculosis and brucellosis.
- Bulk milk is tested for IBR, BVDV and *Salmonella* Dublin every 3 months and for enzootic bovine leucosis every 3 years.

Animal movement control

The success of the eradication of diseases in husbandry in Denmark has ever since the eradication of foot-and-mouth disease by 1970 built on animal movement control above all other measures. Animal movement control meant arrest of import of domestic animals to the country and arrest of domestic animal movement into and out of zones not declared free of disease or control zones surrounding an outbreak of a disease subject to eradication.

Animal movement control historically was governed by Ministry of Agriculture, the Veterinary Services (now Ministry of Family and Consumer Affairs, the Danish Veterinary and Food Administration), and still is subject to legislation and based on principles of livestock identification, registration and traceability as described at

http://www.uk.foedevarestyrelsen.dk/AnimalHealth/Prevention_control_animal_diseases/Livestock_identification_registration_and_traceability/forside.htm

In brief, animal movement implies identification and recording of every movement of individuals of cattle, sheep and goats between premises of registered farms with cloven-hoofed animals. No cloven-hoofed animal must be moved again before 30 days after entry to a farm. The other animals on a farm to which a cloven-hoofed animal has been moved are quarantined on the farm for 7 days unless they are moved directly to slaughter.

Import of animals to Denmark, likewise, historically was governed by the Ministry of Agriculture and still is subject to legislation. Since the implementation of the open market within the European Union in January 1991, import of animals in part has been governed on a voluntary basis by the producer organisations. Thus, the import of cattle currently is regulated also by the Danish Cattle Federation and import of dairy cattle is prohibited according to the dairy quality control program of the dairy industry (Arlagården, 2006). A legitimate import requires certification of freedom for brucellosis, enzootic bovine leucosis and IBR from the country of origin and quarantine for one month in Denmark and serological testing for IBR, BVDV and *Salmonella* Dublin as well as virological testing for BVDV. Although control of animals is done on a voluntary basis failure to comply with the rules will be accompanied by sanctions from the producer organisations. Import of cattle does not take place unattended and compliance with the rules is ensured in all cases.

Access to dairy farms

Since the reintroduction of FMD to Denmark in 1982 and the limited epidemic on the island of Funen that resulted primarily from contact transmissions (Vestergaard, 1982; Christensen et al., 2005b) the awareness of the risks of contact in disease transmission has been very high among Danish farmers. This awareness was further strengthened during the infectious disease eradication programs since the

eighties and entrances to cattle and pig stables are usually marked with a sign of “Entry prohibited unless authorized by the farmer”.

Entry to farms is prohibited for 48 hours after travelling to other countries.

Limitation of access to stables and other measures to limit the risk of contact transmission are formulated in the dairy quality control program of the dairy industry (Arlagården, 2006).

Population containment of Danish dairy cattle

The structure of husbandry in general and dairy cattle farming in particular has undergone a most significant development in Denmark during the past 30 years resulting in a decimation of dairy cattle farms, a 5-6 fold increase in the average number of heads of dairy cows per farm and a reduction by 50% of the dairy cow population as shown in Table 1. This is a development still in progress with undiminished speed.

Table 1. Structural development of dairy cattle farms in Denmark during 1975 to 2006

Numbers x 1000	*1975*	*1980*	*1990*	*2000*	*2005*	*2006*
Dairy farms	63,2	42,4	21,5	9,8	5,9	5,4
Dairy cows	1.106	1.066	769	644	558	548
Cows per farm	17,3	24,5	35,8	65,9	94	101
Imported cows	NA*	NA	NA	NA	NA	0.030

* Data were not available to discriminate between cattle races, but import during the entire period was of the same order of magnitude as for 2006.

Source: Danmarks Statistik and Danish Cattle Federation

Half of dairy cattle farms to day are self supplementary with milk producing cows.

As a result of animal movement control and the restrictions on import of animals imposed by legislation and the producer organisations, there has been very little influx of domestic animals since 1975 to the husbandry in Denmark in general and to the Danish dairy cattle population in particular.

Hygienic measures in production of dietary milk

Milk for human consumption is subject to hygienic measures to prevent transmission of food borne pathogens according to “Bekendtgørelse om fødevarehygiejne” (2006). The regulation are supplementary to and in compliance with Regulation (EC) No 852/2004 and Regulation (EC) No 853/2004 and outlined more specifically in “Vejledning om Mælkekontrol” (2005) and “Vejledning i gennemførelse af visse bestemmelser i forordning (EF) nr. 852/2004 om fødevarehygiejne” (2005). Pasterurization of milk for human consumption by heating to 72^{o}C for 15 seconds is mandatory in Denmark.

Conclusion

It is a result of selection-driven evolution as formulated by Charles Darwin in the concept of survival of the fittest that predators in general do not acquire life threatening infections from their prey. Among a variety of examples that illustrates this principle can be mentioned Suid herpesvirus 1 (SHV-1, Aujeszky’s disease virus) which is inevitably fatal to all animals for which information is available with the exceptions of the host reservoir of porcines and the higher primates. Fatal infections by zoonotic viruses from the prey of man generally are acquired only in cases of consumption of animal species such as rodents (reservoir of monkeypox virus with a mortality of 15% in humans) and primates

(reservoir of simian herpesvirus B, inevitably fatal to humans) which we consider extreme and which is practiced in remote areas of the world, in particular when food resources are limited. It might be argued that an “un-natural” exposure in the form of injections as well as the administration to immunocompromised individuals make such evolutionary reflections irrelevant. Yet, the lack of emerging zoonotic infections during more than 8 decades of injections worldwide of insulin derived from pancreas of bovine and porcine origin supports their validity.

The intersection of the variety of known zoonotic viruses reviewed by Acha and Szyfres (2003) and viruses known to cause mastitis or to possibly be found in milk as reviewed by Wellenberg et al. (2002) is comprised of cowpox virus and pseudocowpox virus. TBE virus can be added since it is known potentially to be transmitted by un-pasteurized goat milk (Gresikova et al., 1975). While the presence of cowpox virus in cows in general and in Denmark in particular is dubious, pseudocowpox virus is known to be ubiquitous – also in Denmark. Since vaccination against variola was arrested due to global eradication of variola in 1977 (WHO, 1980) the species of man has offered itself as an unoccupied host of othopoxviruses, ubiquitous in nature, and this renders poxviruses to be most relevant agents for which safety from contamination in biopharmaceuticals should be assured. The presence of TBE virus in dairy milk in Denmark is considered most unlikely but the possibility can not be ignored. Both poxviruses and TBE virus are assumed to be inactivated by the process of pasteurization mandatory in Denmark but this warrants validation.

Assuming that the review by Wellenberg et al. (2002) is not sufficiently comprehensive with regard to virus species that could accidentally become contaminants of milk, the range of viruses that by introduction could possibly become a threat to cattle in Denmark is reviewed in the present report. Given the population containment of Danish dairy cattle and the measures taken to control the zoosanitary status of husbandry in general and in dairy cattle in particular, the presence of EBL, WNV, EMCV and HEV in dairy cattle in Denmark is considered most unlikely. Should an exotic infection be introduced to Denmark it is unlikely that it should remain unattended. In addition, neither EBL, WNV, EMCV nor HEV is known to be contaminants of milk. Hence, none of these viruses are likely to represent any risk in biopharmaceuticals produced from milk from Danish dairy cows.

In conclusion, the risk of contamination of milk from Danish dairy cows with any known viral pathogen that after pasteurization could represent any threat to humans is very low.

Addressing zoonotic viruses that are unlikely to represent any risk in Danish dairy milk and also the issue of emerging viruses with human pathogenic potential, a combination of virus eliminating steps in the manufacturing process, the nature of function of which should be different, still warrants validation in compliance with current practices in regulatory affairs of biopharmaceuticals. Viruses resistant to physical inactivation and small non-enveloped viruses are considered the most relevant model candidates for which satisfactory elimination should be assured in the manufacturing process using Danish dairy milk of dietary quality as raw material.

References

Acha PN and Szyfres B (2003) Zoonoses and communicable diseases common to men and animals. Third Edition, Volume II: Chlamydioses, Rickettsioses and Viroses. Pan American Health Organization, World Health Organization, Washington DC, USA

Andersen S and Skovgaard T (2004) Viral teat lesions in Danish dairy cattle – incidence and treatment, clinical features and effect on udder health. Veterinært Speciale. Den Kongelige Veterinær- og Landbohøjskole.

Anon. (2006) The current status of BSE and Scrapie in Denmark: February 2006. Ministry of Family and Consumer Affairs, Danish Veterinary and Food Administration.

Anon. (2007) Animal Health in Denmark. Ministry of Family and Consumer Affairs, Danish Veterinary and Food Administration.

Arlagården (2006) Kvalitetsprogrammet Arlagården. www.arlafoods.dk

Bekendtgørelse Nr. 28 af 19. januar 2006. Bekendtgørelse om BVD hos kvæg

Bekendtgørelse Nr. 29 af 19. januar 2006. Om IBR-infektion hos kvæg

Bekendtgørelse Nr. 30 af 19. januar 2006. Om enzootisk kvægleukose.

Bekendtgørelse Nr. 305 af 3. maj 2000. Om brucellose (smitsom kalvekastning) hos kvæg

Bekendtgørelse Nr. 306 af 3. maj 2000. Bekendtgørelse om kvægtubekulose

Bekendtgørelse Nr. 112 af 24. februar 2005. Om Salmonella hos kvæg og svin m.m.

Bekendtgørelse nr. 773 af 6. juli 2006. Bekendtgørelse om fødevarehygiejne. Familie- og Forbrugerministeriet, Fødevarestyrelsen, J. nr. 2006-20-23-01591.

Bollinger O (1877) Tidskrift for Veterinærer.

Burke DS and Monath TP (2001) Flaviviruses. In Fields B, Knipe DM, Howley PM er al. Eds. Virology: 1043-1125.

Burnouf T and Radosevich M (2003) Nanofiltration of plasma-derived biopharmaceutical products. Haemophilia 9(1): 24.

Christensen LS (2005) Kokoppevirus påvist i Danmark. Dansk Veterinærtidskrift 88/10: 31.

Christensen, LS, Nielsen EB, Nowicki J, Andersen J and de Stricker K (2005a), Detection of cowpox virus in Denmark. Ugeskrift for Læger 167/15: 1646-1647.

Christensen LS, Normann P, Thykier-Nielsen S, Sørensen JH, de Stricker K and Rosenørn S (2005b) Analysis of the epidemiological dynamics during the 1982-1983 epidemic of foot-and-mouth disease (FMD) in Denmark based on molecular high-resolution strain identification. Journal of General Virology 86: 2577-2584.

EMEA (2003) EMEA/410/01 rev. 2, section 6.6. Milk and Milk Derivatives.

Esposito JJ and Fenner F (2001) Poxviruses. In Fields B, Knipe DM, Howley PM er al. Eds. Virology: 2885-2921.

Fekuda M, Shaddock JH, Chandler FW and Sanderlin DW (1988) Pathogenesis of rabies virus from a Danish bat (*Eptesicus serotinus*): neuronal changes suggestive of spongiosis. Arch Virol 99: 187-203.

Furuya K, Murai K, Yokoyama T, Maeno H, Takeda Y, Murozuka T, Wakisaka A, Tanifuji M and Tomono T (2006) Implementation of a 20-nm filter in the plasma-derived factor VIII manufacturing process. Vox Sang 92(2): 119-25.

Gresikova M, Sekeyova M, Stupalova S et al. (1975) Sheep milk-borne epidemic of tick-borne encephalitis in Slovakia (Intervirology 5: 57-61.

Jenner E (1798) An inquiry into the causes and effects of the variola vaccinae, a disease discovered in some of the western counties of England, particularly Glouchestershire, and known by the name of cow pox. Reprinted in: Camac LNB, Ed. Classics of medicine and surgery. 1959. New York: Dover: 213-240.

Marennikowa SS, Maltseva NM, Korneeva VL et al. (1979) Outbreak of pox diseases among carnivora (felidae) and edentata. J Infect Dis 135: 358.

De Mattos CA, de Mattos CC and Rupprecht CE (2001) Rhabdoviruses. In Fields B, Knipe DM, Howley PM er al. Eds. Virology: 1245-1277.

McLean et al. (2001) Ann N Y Acad Sci 951: 54-57.

Moss B (2001) Poxviridae: the viruses and their replication. In Fields B, Knipe DM, Howley PM er al. Eds. Virology: 2849-2883..

Müller T, Cox J, Peter W, Schäfer R, Bodamer P, Wulle U, Burow, and Müller W (2001) Infection of a stone marten with European bat lyssavirus (EBL-1) Rabies Bull Eur 25: 9-11.

OIE terrestrial Animal Health Code 2005 article 2.3.13.1, 1a.

ProMED Mail Post (2003) West Nile virus update 2003 – USA (34) 20. Nov.

ProMED Mail post (2007) CJD (new variant) update 2007 (044) 5. February.

Purcell RH and Emerson SU (2001) Hepatitis E virus. In Fields B, Knipe DM, Howley PM er al. Eds. Virology: 3051-3061.

Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstufs.

Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.

Ronsholt L (2002) A new case of European bat lyssavirus (EBL) infection on Danish Sheep. Rabies Bull Eur 26:15.

Skarphedinsson S, Jensen PM, Kristiansen K (2005) Survey of tick borne infections in Denmark. Emerg Infect Dis 11(7): 1055-61.

Tjørnehøj K, Rønsholt L, Fooks AR (2004) Antibodies to EBLV-1 in a domestic cat in Denmark. Vet Rec 155; 571-2.

Vejledning i gennemførelse af visse bestemmelser i forordning (EF) nr. 852/2004 om fødevarehygiejne. Europa kommissionen, Generaldirektoratet for Sundhed og Forbrugerbeskyttelse. Bruxelles, 21. december 2005.

Vejledning om mælkekontrol. J. nr. 2005-20-23-01432. Fødevarestyrelsen, 23. december 2005.

Wellenberg GJ, van der Poel, WHM and van Oirschot JT (2002) Viral infections and bovine mastitistis: a review. Veterinary Microbiology 88: 27-45.

Westergaard JM (Ed.) (1982) Report on the eradication of foot-and-mouth disease on the islands of Funen and Zealand, Denmark 1982. The Danish Veterinary Service, Copenhagen, Denmark.

World Health Organization (1980) The global eradication of smallpox. Final report of the Global Commission for the Certification of Smallpox Eradication. Geneva: World Health Organization.

World Health Organization (2003) WHO guidelines on transmissible Spongiform Encephalopathies in relation to biologic and pharmaceutical products: page 8. Geneva: World Health Organization.

Vos A, Müller T, Cox J, Neubert L and Fooks AR (2004) Susceptibility of ferrets (*Mustela putorius furo*) to experimentally induced rabies with European bat lyssavirus (EBLV). J Vet Med B 51: 55-60.

Vos A, Müller T, Neubert L, Zurbriggen A, Botteron C, Pöhle D, Schoon H, Haas L and Jackson AC (2004) J Vet Med B 51: 327-332

Naional Food Institute
Tecnical University of Denmark
Mørkhøj Bygade 19
DK - 2860 Søborg
Denmark

P: +45 72 34 70 00
F: +45 72 34 70 01
www.food.dtu.dk

ISBN: 978-87-92158-04-8